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Assessing differences in cardio-respiratory fitness with respect to maturity status in highly trained youth soccer players

Abstract

Purpose: Examine differences in measures of cardio-respiratory fitness and determinants of running economy with respect to maturity status, in a group of highly trained youth soccer players.

Methods: Twenty-one highly trained youth soccer players participated. On separate visits, players' peak oxygen uptake, running economy, at three different speeds ($8\text{km}\cdot\text{h}^{-1}$, 80%GET and 95%GET), and pulmonary oxygen uptake kinetics (VO_2 kinetics) were determined. Players also performed a Yo-Yo Intermittent Recovery test level 1 (Yo-Yo IR1). Players were categorised as either 'pre-PHV' or 'mid-PHV' using the measure of maturity offset. Independent *t*-tests and Cohen's *d* effect sizes were then used to assess differences between groups.

Results: The mid-PHV group were significantly taller, heavier and advanced in maturity status. Absolute measures of $\text{VO}_{2\text{peak}}$ were greater in the mid-PHV group, however, when expressed relative to body mass, FFM and theoretically derived exponents, $\text{VO}_{2\text{peak}}$ values were similar between groups. Pre-PHV group presented a significantly reduced VO_2 response, during relative submaximal running speeds, when theoretically derived exponents were used, or expressed as % $\text{VO}_{2\text{peak}}$. VO_2 kinetics (*tau*) were faster during a low (standing) to moderate (95% GET) transition in the pre-PHV group. Yo-Yo IR1 performance was similar between groups.

Conclusion: While measures of $\text{VO}_{2\text{peak}}$ and Yo-Yo IR1 performance are shown to be similar between groups, those classed as Pre-PHV display a superior running economy at relative submaximal running speeds and faster *taus* during a low-moderate exercise transition, than their more mature counterparts.

25 **Introduction**

26 The growing professionalism of sport has resulted in an advancement in the application of early
27 specialization and systematic training of talented young athletes, with the aim of achieving elite
28 professional status (26). Consequently, a large emphasis is placed on the talent identification
29 and talent development of young soccer players (38). This has resulted in research which aims
30 to identify, examine and analyse the key physical and physiological characteristics of elite
31 youth soccer players that are associated with superior soccer performance (38). However,
32 confounding variables of growth and maturation complicate the analysis and interpretation of
33 these measures within youth populations as well as the provision of coaching and training
34 prescription toward these diverse populations. Indeed, both physiological and anthropometric
35 changes can occur during adolescence, particularly during rapid periods of growth and
36 maturation, these changes then impact upon physical performance and confound the issues of
37 talent development and identification (17).

38

39 Currently, there is a plethora of research examining the impact of maturational differences in
40 anaerobic fitness characteristics (speed, acceleration, jump power, etc.) (12, 17), highlighting
41 the physical dominance of players who are at an advanced stage of maturity (26, 12). Although
42 youth soccer players will compete within their respective age groups (same chronological age),
43 the impact of growth and maturation can result in large variations in skeletal age within male
44 youth soccer players aged between 10-18 years old. Subsequently, those individuals which are
45 of an advanced maturity status will often appear as the taller, heavier and more physically
46 developed individuals. This can often result in a selection bias towards players of an advanced
47 maturity status, due to their superior levels of speed, power and strength (26, 27), leading to
48 talented, but physically less mature players not attaining their full potential and dropping out
49 of the game (12).

Research has highlighted the dominance of early maturing individuals in anaerobic fitness characteristics (27). The energy provision required for competitive soccer performance, however, is predominantly derived from aerobic energy sources (19). While an improved ability to activate anaerobic energy resources will result in superior performance in one-off explosive actions during match-play (sprints), an enhanced ability to utilise aerobic energy resources will (theoretically) result in an improved tolerance to fatigue and an ability to maintain levels of high intensity activities, and therefore maintain physical performance for a prolonged period of time (33). Therefore, while the less mature player may be unable to metabolically activate anaerobic energy sources, they will however, have the capacity to sustain high intensity activities via primarily aerobic energy pathways (33). Yet, there is little information regarding the impact of maturation with respect to detailed measures of cardio-respiratory fitness, in highly trained youth soccer players. Moreover, recent research has highlighted the importance of appropriate scaling to successfully accommodate the nonlinear relationship between body size descriptors and VO_{2peak} (10, 25, 1). Adopting the traditional ratio-standard method ($mL \cdot kg^{-1} \cdot min^{-1}$) results in an adjusted VO_{2peak} which is not independent of body size, therefore research advocates the use of allometric scaling, when interpreting VO_2 data, to remove the confounding influence of body size, in individuals at different stages of maturation (10).

Therefore, the purpose of this study was to examine inter-group differences in ratio-standard and allometrically-adjusted measures of submaximal and peak cardio-respiratory fitness and determinants of running economy with respect to maturity status, in a group of highly trained youth soccer players. It was hypothesized that, when appropriately scaled, measures of VO_{2peak} would be unaffected by maturity status. Players of a lower maturity status, however, would present a reduced VO_2 response during all submaximal running speeds, when appropriately

scaled. Similarly, it was hypothesised that less mature players would display superior VO_2 kinetics (faster *taus*), than their more mature counterparts.

Methods

Participants and Anthropometry

Twenty one highly trained youth soccer players aged between 12 and 14 years volunteered to participate in this study. All participants were outfield players from the same Category One Premier League Football Academy. Prior to the commencement of the study, all players completed medical health questionnaires and training history questionnaires. Table 1 displays all anthropometric and screening measures. Maturity status was quantified using self-assessment Tanner Stage method (30, 36) and maturity offset (31). Sum of 4 skinfolds was assessed using Harpenden skinfold calipers (Cranlea, Birmingham, UK) to measure skinfold thickness at the biceps, triceps, subscapular and superilliac according to the procedures outlined by ISAK (28). Percent body fat was then estimated using the equation of Siri (35), this information was then subsequently used to estimate individuals' fat free mass (FFM). Players and their parents were informed about all procedures and requirements involved in the study before opting to provide written informed consent and assent from parents and participants, respectively. Ethical approval was granted from the local university ethics committee.

Calculation of Maturity Offset

Players were categorised into pre-PHV and mid-PHV groups using the calculation of maturity offset (31). Measurements obtained to gain an estimate of each individual's maturity offset included stretch standing stature and stretch sitting stature, to the nearest mm (Seca 217, Birmingham, United Kingdom) as well as body mass, to the nearest 0.1 kg (Seca 761, Birmingham, United Kingdom). As described by Mirwald et al. (31) two measurements were

taken for each anthropometric measure, with a third required if the first two measures differed by more than 4 mm for measures of stature and 0.4 kg for weight. For each anthropometric measure these were then averaged. All measurements were obtained by a sport practitioner experienced in the assessment of such procedures. This data was then inputted into a specially designed Microsoft Excel Spreadsheet which employed the maturity offset equation of Mirwald et al. (31) to provide an estimated value for the years from peak height velocity (PHV) for each individual.

Once the maturity offset was known, participants were classified as either pre-PHV or mid-PHV. Players who were ≥ -4.0 and < -1.0 year away from their respective period of PHV were classified as pre-PHV ($n = 10$), while those who were between ≥ -1.0 and $< +0.99$ years from their predicted PHV were classified as mid-PHV ($n = 11$). The maturity offset measure gives an indication to an individual's maturity status, as such, those who were classified as mid-PHV were at an advanced stage of maturation, when compared to their counterparts who were classified as pre-PHV.

Table 1: Anthropometric and screening measures ($n = 21$)

Insert Table 1 Here

Study Design

Players' anthropometric measurements were collected first, followed by laboratory then field tests. The players visited the laboratory on 4 separate occasions. During the first visit, players performed an incremental, ramp treadmill protocol for the assessment of players' gaseous exchange threshold (GET), VO_{2peak} and the speed corresponding to 60% of the difference between GET and VO_{2peak} ($60\%\Delta$). Players then returned to the laboratory for a second time,

following a minimum of 24 hours of recovery. On the second visit, players were required to run for 4 min, at progressive sub-maximal intensities ($8\text{km}\cdot\text{h}^{-1}$, 80%GET and 95%GET), with 2 min passive recovery between speed increments. Players returned to the laboratory on two further occasions, each following a minimum of 24 hours of recovery. These two visits to the laboratory were identical and required the participant to complete a work-to-work protocol (rest - 95%GET – 60% Δ) on a motorised treadmill for the assessment of their pulmonary oxygen uptake kinetics (VO_2 kinetics). Following all laboratory tests and a minimum of 48 hours recovery, players completed a maximal Yo-Yo Intermittent Recovery test level 1 (Yo-Yo IR1) (6). For each participant, all testing was completed at the same time of day (± 2 hours), with the ranges for room temperature, humidity and pressure corresponding to $18.7 - 22.8^\circ\text{C}$, 62 - 63 % and 1011 - 1021 mmHg respectively, for laboratory testing.

Assessment of Peak Oxygen Uptake and Gaseous Exchange Threshold

Upon arrival to the laboratory and following the necessary screening procedures, participants were fitted with a Polar Heart rate monitor (Polar Electro, Kempele, Finland) and face-mask (Hans Rudolph, Hans Rudolph, Kansas City, USA), which was connected to an online gas analysis system (Cortex MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany). The online gas analyser was calibrated prior to each visit according to the manufacturer's instructions, using a known gas concentration and a 3-L syringe for manual volume calibration of the ventilation sensors. Prior to each test, participants completed a standardised 10 min warm up consisting of 4 min running at $8\text{km}\cdot\text{h}^{-1}$, 3 min of dynamic stretches (mainly focusing on the lower body) and a further 2 min of sub-maximal running before a final minute for the participant to perform their own stretches. Following this, and a full description of the test and safety procedures, participants began to run at a speed of $8\text{km}\cdot\text{h}^{-1}$ at a 1% incline (21) on a motorised treadmill (HP Cosmos, Pulsar, Sportgerate GmbH, Nussdorf, Germany). The speed

of the treadmill was increased by $1\text{km}\cdot\text{h}^{-1}$ every two minutes, this continued until participants reached 90% of their age predicted heart rate max ($207 - (0.7 \times \text{age})$) (16). At this point, the treadmill speed remained constant whilst the incline of the treadmill was increased by 1% every minute until volitional exhaustion; this procedure was employed to avoid over-striding and potential early termination and inaccurate assessment of participant's $\text{VO}_{2\text{peak}}$. This method has been successfully used before to elicit $\text{VO}_{2\text{peak}}$ during treadmill running in young athletes (40). Rolling 15 sec averages were calculated for the final minute of the test, with the highest 15 sec average during the test being regarded as $\text{VO}_{2\text{peak}}$ (5).

The gaseous exchange threshold (GET) was identified using the V-slope method (V_E (ordinate), VO_2 (abscissa). Two regression lines were created based upon the relationship between VCO_2 and VO_2 . The intercept point between the two regression lines was then visually identified, with the VO_2 value at the intercept (GET) being extrapolated to the abscissa. To identify the speed at GET, a regression line was formulated for VO_2 and running velocity, for each individual. The GET was assessed by two individual researchers (experienced in the detection of GET), demonstrating 81% agreement (17 out of 21). For the remaining four participants, a third researcher, also experienced in the detection of GET, was approached to verify the GET (15). The individual's VO_2 at GET was then inputted into the individual's respective regression equation to calculate the running velocity at GET. The V-slope method has been shown to be a reliable method for detecting and identifying the gaseous exchange threshold in children (15).

Assessment of Running Economy

On the subsequent visit to the laboratory, and following a minimum of 24 hours recovery, participants' RE was assessed at three progressive sub-maximal intensities (one absolute:

8km·h⁻¹ and two relative: 80%GET and 95%GET exercise intensities), with 2 min passive recovery between each intensity. Prior to testing, participants were fitted with a Polar Heart rate monitor and face-mask, which was connected to the pre-calibrated online gas analysis (Cortex) system. Following a standardised 10 min warm-up and a full description of the test procedures, participants began to run at the lowest exercise intensity (for 3 of the participants the velocity at 80%GET was lower than 8km·h⁻¹), with the gradient of the treadmill set at 1% (21). Participants ran at this speed for 4 min, ensuring that a steady state was maintained for the final minute (8). A 2 min passive recovery was then permitted, in which participants' heart rate recovered to within 10% of pre-exercise levels. Pilot testing demonstrated that a 2 min passive recovery was shown to provide sufficient time for players to recover, ensuring there were no ensuing effects on the subsequent exercise bouts. During this recovery period, the treadmill speed was increased to the next progressive speed (higher intensity of 8km·h⁻¹ or 80%GET). Again, participants exercised at this speed for 4 min, attaining a steady state, before undertaking a further 2 min recovery. Following the final 2 min of recovery, participants completed another 4 min period of sub-maximal running at the highest intensity (95%GET). Extraction of the appropriate measures for assessing RE included applying rolling 15 sec averages for the final minute of each sub-maximal exercise intensity to ensure that a steady state had been attained. Current results revealed that all participants (100%) attained a steady state (< 2 mL·kg⁻¹·min⁻¹, in the final minute of exercise) for each sub-maximal exercise intensity. Following the identification of a steady state, RE was defined as the average VO₂ during the final minute of each exercise bout (8). Relative oxygen consumption (VO₂) was obtained for each exercise intensity as well as determinants of RE: minute ventilation (V_E) and ventilatory equivalent (V_EVO₂).

Measures of VO_2 in both the maximal graded exercise test and the assessment of running economy, for each sub-maximal running speeds, were normalized using 1) ratio-standard scaling to body mass ($\text{mL}\cdot\text{kg BM}^{-1}\cdot\text{min}^{-1}$) and fat free mass ($\text{mL}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$), 2) allometric scaling, using theoretically derived exponents (9, 25; $b = 0.67$ and $b = 0.75$) and, 3) a measure relative to participants' $\text{VO}_{2\text{peak}}$ ($\% \text{VO}_{2\text{peak}}$).

Assessment of oxygen uptake kinetics

On the two remaining visits to the laboratory, each participant completed a series of exercise transitions to a higher intensity on the motorised treadmill. Each series consisted of 3 min unloaded (standing), 4 min running at an intensity equivalent to 95% GET (moderate intensity) and a run to exhaustion at an intensity equivalent to 60% Δ (severe intensity). Prior to the test, participants were familiarised with the transition to each speed given sufficient time to practise until they felt comfortable with both transitions (unloaded – moderate and moderate – severe). Following familiarisation to the speed transitions, participants were fitted with a Polar Heart rate monitor and face mask, which was connected to an online expired gas analysis system. During the test, verbal encouragement was provided throughout for participants to continue for as long as possible, however no visual feedback relating to exercise duration, was given to the participants during the test.

For the unloaded to moderate transition the treadmill was set at the relevant intensity for each individual, while the participant straddled the treadmill. The participant was then given a 10 second countdown at the end of the unloaded phase, at which point they lowered themselves onto the moving treadmill and began exercising. For the transition from moderate to severe intensity the participant remained running on the treadmill. The time taken for each exercise transition was in all cases < 5 seconds, thus having minimal effects on the VO_2 kinetic response

as this would be contained within the cardiodynamic phase of the oxygen uptake response to an increase in intensity (40).

Mathematical modelling of oxygen uptake kinetics

Prior to the modelling of the VO_2 kinetics errant breaths (coughing, swallowing, sighing, etc.) were removed from the raw data set so as not to distort or skew the underlying physiological response. Errant breaths were defined as a breath that was different to the mean of the surrounding four breaths by more than four times the standard deviation of the same surrounding four data points (22). Both data-sets from each stage (unloaded – moderate and moderate – severe) were time aligned and ensemble averaged to enhance the underlying physiological response characteristics for all intensities. Each ensemble average was then linearly interpolated second-by-second prior to the modelling process. Custom written software in Microsoft Excel, using the Solver function, was utilised for all modelling processes.

Pulmonary oxygen uptake kinetics for the unloaded to moderate and moderate to severe transitions were modelled separately, due to the difference in the characteristics of the kinetic response for each increase in intensity. For both, unloaded – moderate and moderate – to severe transitions, the goal of the modelling process was to isolate the fundamental phase (39) of VO_2 kinetic response (equation 1). To eliminate the influence of the cardiodynamic phase on the modelling of VO_2 kinetics, the initial 20 seconds from the unloaded to moderate phase and initial 15 seconds of the moderate to severe phase were removed prior to the modelling process. A time delay of 20 seconds is often employed to accommodate the cardiodynamic phase (39); however, a 15 second time delay was adopted for the moderate to severe transition due to elevated baseline blood flow incurred from the prior moderate intensity (7). Following the

cardiodynamic phase, $\dot{V}O_2$ kinetics were assumed to develop initially via a single exponential term (fundamental phase), following a delay relative to the start of exercise of the form:

$$\dot{V}O_{2(t)} = \dot{V}O_{2(b)} + A_{\dot{V}O_2} \times (1 - \exp^{-(t-TD)/\tau}) \quad [1]$$

Where $\dot{V}O_{2(b)}$ is the baseline $\dot{V}O_2$, which was taken as the last 30 seconds of oxygen uptake during 3 minutes unloaded phase. $A_{\dot{V}O_2}$ represents the asymptotic amplitude of the fundamental component of the response; τ is the time constant of the fundamental component and TD is the time delay similar, but not equal to the cardiodynamic-fundamental phase transition time. For the unloaded – moderate transition, the fundamental phase was considered *a priori* to encapsulate the entire 4-minute transition since exercise was undertaken below the GET. For the moderate – severe transition, the fitting strategy was designed to identify the onset of the “slow component” of the response to exercise, and thus isolate the fundamental component. Starting at 60 s, the fitting window was therefore widened by 1 s until the end of exercise with the time constant and reduced chi-square value of the curve of best fit for each time window plotted against time. The onset of the slow component could then be identified as the coincident point at which a plateau or minima in the value of τ and a minima in chi-square, followed by a progressive increase in these values, could be determined as its value becomes affected by the slow component. The time at which this occurred was used as the optimal fitting window with which to determine the kinetics of the fundamental phase of $\dot{V}O_2$ kinetics. The phase III of oxygen uptake (steady state in the unloaded – moderate transition) was taken as the sum of $\dot{V}O_{2(b)}$ and $A_{\dot{V}O_2}$. The amplitude of the slow component during the moderate – severe transition was calculated as $\dot{V}O_2$ at exhaustion minus the phase III $\dot{V}O_2$.

Yo-Yo Intermittent Recovery Test Level 1

For the Yo-Yo IR1 test, cones were placed 20 m apart, with a 5 m recovery zone marked out at one end. The Yo-Yo IR1 test requires participants to run 2 x 20 m shuttle runs at increasing speeds, interspersed with 10 seconds of active recovery. The pace of the test was controlled by audio signals emitted from a CD player (Sony CFD-V7, Sony, Tokyo, Japan). For the maximal Yo-Yo IR1 test players were required to run until volitional termination of the test or, when they have twice failed to meet the designated cones in time with the audio signal, at which point they are removed from the test. The test score is the distance covered at the point they withdraw from the test. During the test, players were allowed to consume fluids ad libitum. Current findings support the use of the Yo-Yo IR1 test as a valid measure of physical performance, associated with soccer match-play, particularly within youth populations (6).

Statistical Analysis

A Shapiro Wilks test found the data to be normally distributed, therefore parametric statistical calculations were applied. Following this, independent *t*-tests were conducted to assess the differences between pre-PHV and mid-PHV groups, in anthropometric measures and measures of cardio-respiratory fitness. Differences between groups were also compared using Cohen's *d* effect sizes (ES) and thresholds (<0.5 = small; 0.5-0.8 = moderate; >0.8 = large) to aid interpretation. Additionally, where appropriate, a qualitative descriptor, used to aid practical inferences, was assigned to the following quantitative chances of benefit: 25-75% = benefit possible; 75-95% = benefit likely; 95-99% = benefit most likely; >99% = benefit almost certain. All statistical analysis was performed using SPSS version 21.0 (IBM SPSS statistics for Windows, IBM, Armonk, New York) and Microsoft Excel (Microsoft Excel 2013, Microsoft, Redmond, Washington) with the level of significance (alpha) set at 0.05.

Results

Differences in anthropometric and descriptive measurements revealed significant differences and large effect sizes for stature, body mass, fat free mass and maturity offset, with the mid-PHV group presenting greater values than the pre-PHV group, despite being of similar chronological age (Table 2). Furthermore, years spent training also revealed significant and large effect sizes between groups, but with the pre-PHV group demonstrating greater values than the mid-PHV group, meaning that those identified as pre-PHV had reported to have spent a greater amount of years of systematic training at a high level than the mid-PHV group.

Table 2: Differences in anthropometric and descriptive characteristics between those identified as pre- and mid-PHV, in highly trained youth soccer players.

*** Insert Table 2 Here***

While absolute measures ($\text{L} \cdot \text{min}^{-1}$) of $\text{VO}_{2\text{peak}}$ were shown to be greater in the mid-PHV group, the difference between the two groups was eradicated when expressed relative to body mass, fat free mass, as a $\% \text{VO}_{2\text{peak}}$ or when using theoretically derived exponents (Table 3). In addition, a moderate effect size was revealed for the velocity at GET, with the mid-PHV group demonstrating a faster velocity when running at GET.

Limited differences were evident between the two groups for both, measures of cardio-respiratory fitness and determinants of running economy when running at an absolute speed of $8\text{km} \cdot \text{h}^{-1}$. Similarly, no differences in running economy were evident for each submaximal running speed when VO_2 was expressed in relation to body mass or fat free mass. For both the relative submaximal exercise intensities, however, the pre-PHV group presented significantly superior levels of running economy, when VO_2 data was scaled using theoretically derived

exponents. In support of this, the pre-PHV group also demonstrated a reduced fractional utilisation ($\% \text{VO}_{2\text{peak}}$) for the same relative submaximal exercise intensities (Table 3). Figure 1 displays a representative plot of the VO_2 for each of the three sub-maximal running speeds and during each of the 2 min passive recovery phases.

Table 3: Differences between those identified as pre-PHV and mid-PHV, in measures of cardio-respiratory fitness during the maximal graded exercise test and during both absolute ($8\text{km}\cdot\text{h}^{-1}$) and relative (80% and 95% GET) submaximal running speeds, using different methods for normalizing VO_2 .

Insert Table 3 Here

Figure 1: Representative plot of the VO_2 for each of the three sub-maximal running speeds and during each of the 2 min passive recovery phases

Insert Fig 1 Here

Measures of VO_2 kinetics revealed a significant difference for the absolute amplitude ($\text{L}\cdot\text{min}$) and a large effect size for the phase II τ , during the low – mod transition, with the pre-PHV group demonstrating faster oxygen uptake kinetics than the mid-PHV group. Figure 2 displays a representative plot of the pulmonary oxygen uptake kinetics during the work-to-work protocol.

Figure 2: Representative plot of the pulmonary oxygen uptake kinetics during the work-to-work protocol, with the respective unloaded - moderate and moderate - severe time constants (τ s) (Doncaster et al., 2016).

Insert Fig 2 Here

Finally, performance in the Yo-Yo IR1, a measure of soccer-specific endurance, was similar between the two groups (mid-PHV: 1681 ± 304 vs. pre-PHV: 1572 ± 360 m, $P = 0.49$, $d = 0.33$), revealing a small effect size.

Discussion

The main finding from the current study was that highly trained soccer players of advanced maturity status do not outperform their less mature counterparts, in key measures of cardio-respiratory fitness and determinants of running economy. Instead, results show that players identified as less mature (pre-PHV) display enhanced levels of cardio-respiratory fitness, particularly when accounting for differences in body size.

In line with the existing literature (24, 26, 27), anthropometric and descriptive characteristics found those players of an advanced maturity status (mid-PHV) to be physically taller and bigger, presenting both a larger stature, body mass and estimated fat free mass than their less mature counterparts (pre-PHV) of a similar age. The pre-PHV group, however, reported a greater amount of training experience at a high level than their more mature counterparts. Despite those identified as mid-PHV displaying a significantly greater absolute measure of $\text{VO}_{2\text{peak}}$, when these data were expressed relative to: body mass ($\text{mL.kg BM}^{-1}.\text{min}^{-1}$), fat free mass ($\text{mL.kg FFM}^{-1}.\text{min}^{-1}$) or when using theoretical exponents ($\text{mL.kg}^{-0.67}.\text{min}^{-1}$ and $\text{mL.kg}^{-0.75}.\text{min}^{-1}$), the differences between the two groups were eliminated (Table 3). Similarly, performance during the Yo-Yo IR1 test was comparable between the two groups, revealing only a small effect size in the favour of the mid-PHV group. Results, however, do propose a preference for oxidative metabolism in less mature players (pre-PHV), when compared to their more mature counterparts (mid-PHV). Indeed, when running at relative sub-maximal intensities (80% & 95% GET), normalized VO_2 values using theoretically derived exponents

($b = 0.67$ and $b = 0.75$), the pre-PHV group revealed a significantly reduced VO_2 response for a given relative sub-maximal intensity. In support of this, the pre-PHV group also presented a reduced fractional utilisation ($\%\text{VO}_{2\text{peak}}$) when running at relative sub-maximal intensities (80% & 95% GET), as well as faster VO_2 kinetics during low – moderate exercise transitions (τ), in comparison to their more mature counterparts.

The results obtained from the tests employed within the current study would appear to be indicative of highly trained youth soccer players. Relative measures of $\text{VO}_{2\text{peak}}$ in the current study are in-line with those of Cunha et al. (9), who reported values of $59.6 \pm 4.3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in pubescent (Age: 13.4 ± 1.0 yr, Body Mass: 62.5 ± 9.9 kg) soccer players and Le Gall et al. (24), who reported values of $59.2 \pm 3.2 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ in U14 international players (Age: 13.4 ± 0.4 yr, Body Mass: 52.5 ± 9.9 kg). Similarly, players' performance in the Yo-Yo IR1 test within the current study is greater than figures reported within the literature for players of a similar age and standard of performance. Indeed, Deprez et al. (11) and Deprez et al. (13) reported scores of 1319 ± 366 m and 1270 ± 440 m for U13 high level youth soccer players, respectively. Deprez et al. (11), however, did separate their cohort of U13 players into low, average and high performers and reported scores of 886 ± 114 m, 1357 ± 100 m and 1714 ± 145 m in the Yo-Yo IR1, respectively. In this regard players' performance in the current study of 1682 ± 304 m for the mid-PHV group and 1572 ± 360 m for pre-PHV group, could be regarded as above average (11). Nevertheless, the current data demonstrates that the players recruited within this study are indicative and therefore representative, of highly trained youth soccer players.

Current results are in accordance with Cunha et al. (9) who also found that while those of advanced maturity present higher absolute values ($\text{L}\cdot\text{min}^{-1}$) of $\text{VO}_{2\text{max}}$, when expressed relative

to body mass ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) differences in measures of $\text{VO}_{2\text{peak}}$ are removed (10, 25). As in the present study, Cunha et al. (9) also analysed their data using theoretical exponents ($b = 0.67$ and $b = 0.75$), as well as their own experimentally derived exponent ($b = 0.90$), using linear regression analysis, to scale participants' VO_2 response. While Cunha et al. (9) reported a significant difference between pre- and post-pubescent youth soccer players, they also reported that differences between groups could not be attributed to biological maturation in the multiple linear regression analysis. Initial findings (2, 3, 4), examining the impact of maturation on $\text{VO}_{2\text{max}}$ reported that maturation positively affected measures of $\text{VO}_{2\text{max}}$, however, authors only expressed measures of $\text{VO}_{2\text{max}}$ in absolute terms. In line with the present findings, recent research (9, 10, 25) advocates the need for appropriate analytical procedures (scaling techniques using lower-limb muscle volume) when assessing differences in VO_2 within heterogeneous populations with respect to maturity status. Appropriately interpreted measures of $\text{VO}_{2\text{peak}}$ are unaffected by maturation in highly trained youth soccer players. Whether or not this has implications for physical performance during soccer match-play requires further investigation.

Present results suggest a reduced oxygen cost for a given relative submaximal running intensity, in players identified as pre-PHV, when compared to their more mature counterparts. There is a paucity of research to date which has examined differences in running economy between individuals of different maturity status, particularly in team sport athletes. Segers et al. (34), reported no differences in the oxygen cost (expressed in absolute terms, using allometric scaling ($b = 0.59$) or as a $\% \text{VO}_{2\text{peak}}$) of running between youth soccer players who were identified as either 'early' or 'late' maturers, when running at absolute speeds of 8, 9.5 and $11 \text{ km} \cdot \text{h}^{-1}$ on a treadmill. The use of absolute running speeds however, in the study of Segers et al. (34) may be inappropriate for youth populations as it is likely to result in different internal

responses, leading to large inter-individual variability and subsequently increased standard deviations, masking any inter-group differences (32). The use of relative running intensities however, will reduce the inter-individual variability and is a more appropriate method by which youth soccer players' running economy can be assessed, in relation to maturity status. Indeed, the improved levels of running economy and fractional utilisation at relative submaximal intensities, in the present study, elucidates to an improved ability to utilise the aerobic metabolism in those identified as pre-PHV, potentially compensating for the undeveloped anaerobic metabolism (24, 27, 33), within the pre-PHV group. Ratel (33) suggests that younger populations (e.g. children) have a reduced capacity for anaerobic activity (lower concentrations of glycolytic enzyme activity [phosphofructokinase], lower muscle mass, reduced percentage of type II muscle fibres) but an improved capacity for aerobic energy production (increased oxidative phosphorylation enzyme activity [citrate synthase], increased percentage of type I muscle fibres). The extent to which these physiological mechanisms are affected by high levels of systematic team sport training requires further investigation.

Another means of evaluating an individual's aerobic fitness is via the assessment of VO_2 kinetics. The relative contribution of oxidative and non-oxidative energy supply during exercise are dependent upon the characteristics of the VO_2 response to exercise, and is regarded as an important indicator of aerobic function that influences an individual's capacity to perform (22). Current results provide evidence to suggest superior VO_2 kinetics (faster *taus*) during a low to moderate exercise transition, but not during a moderate to severe transition, in youth soccer players identified as pre-PHV. Indeed, faster VO_2 kinetics (*tau*) during low to moderate exercise transitions have been shown to be correlated with the volume and maintenance of high speed activities during youth soccer match-play (14). With faster *taus* relating to a greater volume and an improved ability to maintain high speed activities during youth soccer match-

play. Furthermore, research into adolescent male and female soccer players has demonstrated faster VO_2 kinetics in soccer trained individuals in comparison to their untrained counterparts (29, 37). Current results also support previous research which highlights faster *taus* in younger populations, with such measures becoming progressively slower (i.e. larger *taus*) as individuals transition from childhood, through adolescence and then to adulthood (23). Nevertheless, it should be acknowledged that players' VO_2 kinetics profiles were averaged from two transitions and that, where possible, multiple transitions should be encouraged as this will improve confidence in the model parameter estimates (e.g. *tau*) (22).

In contrast to measures of anaerobic fitness components, such as speed, power and strength (26, 27), where those of an advanced maturity status are shown to excel, measures of aerobic fitness are shown to be either, similar between groups of differing maturity status or favour less mature individuals, when appropriately scaled (9, 10, 25). This is supported by the present result, which show no differences between pre- and mid-PHV groups in measures of soccer-specific endurance (Yo-Yo IR1), as well as the absence of any relationship between players' maturity offset and Yo-Yo IR1 performance ($r = 0.16$, $P > 0.05$). As noted by Malina et al. (27) the years spent in formal training was a significant contributor to intermittent endurance capacity (measured via the Yo-Yo IR1) in a cohort of 13-15 yr old, high level youth soccer players. Therefore, as those in the pre-PHV group reported to have spent significantly more years training than their more mature counterparts (pre-PHV: 5.9 ± 1.8 vs. mid-PHV: 3.7 ± 2.0 yr, $P < 0.05$, $d = 1.16$), any maturational differences between the two groups, in the current results, may have been offset by the greater level of experienced training within the pre-PHV group. Furthermore, Lovell et al. (26) reported trivial and small effect sizes in the Multi-stage fitness test (MSFT), between players born in the first quarter of the year (Sept-Nov) and players born in the final quarter of the year (June-Aug) in U13-U16 English academy youth soccer

471 players. Although, chronological age rather than maturity status was used to separate groups in
472 the study of Lovell et al. (26).

473
474 Despite the consensus for appropriate allometric scaling within the literature, and in particular
475 the support for experimentally derived components (9, 25), the current study only employed a
476 small sample size. Indeed, research suggests that relatively large sample sizes are required to
477 lower statistical inference errors, and that factors such as ‘quality’ and ‘quantity’ of the data,
478 as well as analysis method (e.g. nonlinear regression or linear regressions) are likely to
479 influence the allometric scaling exponent beta estimation (18). Given the small samples (pre-
480 PHV: $n = 10$, mid-PHV: $n = 11$) within the current study, an experimentally observed exponent,
481 for allometric scaling, was not possible. As a result, theoretical exponents ($b = 0.67$ and $b =$
482 0.75), estimates of Fat Free Mass and a fractional utilisation measure ($\%VO_{2peak}$) were
483 employed to assess highly trained youth soccer players’ VO_2 responses, in relation to maturity
484 status (i.e. pre-PHV vs. mid-PHV). Future research, however, where possible, should look to
485 develop their own experimentally observed exponents, for allometric scaling. Furthermore,
486 limitations associated with the calculations used for estimating body fat percentage, and
487 therefore FFM should also be acknowledged. Mainly the appropriateness of the algorithms
488 used (35), to the elite youth soccer players should be considered. Nevertheless, in the absence
489 of population specific algorithms, previous research examining body composition in
490 professional soccer players have employed these methods (20). Despite these limitations the
491 current study extends and improves upon the existing research by examining differences in
492 distinct measures of cardio-respiratory fitness, and determinants of running economy with
493 respect to maturity status and in a group of highly trained youth soccer players, in which there
494 has been an attempt to account for the effects of body size on VO_2 .

While the present results do advocate the need for an enhanced aerobic capacity in highly trained youth soccer players that are identified as pre-PHV. They also demonstrate that, youth academy soccer players demonstrate high levels of soccer-specific endurance (Yo-Yo IR1), irrespective of maturity status (41). An enhanced capacity for aerobic metabolism, in those identified as pre-PHV may potentially transcend into levels of physical performance during high intensity intermittent exercise that are in comparison to their more mature counterparts. Nevertheless, coaches should be aware that potential differences in physical performance between players identified as pre-PHV or post-PHV are likely to be a consequence of anaerobic fitness capabilities and not aerobic fitness capabilities (24, 26, 41). An emphasis on such anaerobic fitness capabilities (e.g. strength and speed), may be one reason why there is a selection bias toward players of an advanced maturity status within these age categories (12-14 yrs old), often referred to as the relative age effect within soccer (12, 17, 26).

Conclusion

In summary, current findings provide further evidence for differences in anthropometric and descriptive measures between players identified as pre-PHV and players identified as post-PHV, despite similar chronological age. The present data, however, also demonstrates that players of an advanced maturity status (mid-PHV) do not outperform their less mature (pre-PHV) counterparts in a range of measures and determinants of aerobic fitness. Rather, the current data would suggest that those identified as pre-PHV have a superior ability to utilise aerobic energy pathways during relative submaximal intensities, while also displaying faster VO_2 kinetics (*tau*) when transitioning from a low to moderate exercise intensity. Despite the potential for improved capacity for oxidative metabolism in the pre-PHV group, soccer-specific endurance performance (Yo-Yo IR1) was similar between the two groups.

Nevertheless, these enhanced levels of cardio-respiratory fitness may partly explain the comparable levels of high intensity-intermittent performance during the Yo-Yo IR1 test.

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643 **Table 1:** Anthropometric and screening measures (*n* =21)

Variable	Mean ± Standard Deviation	90% Confidence Limits
Age (y)	13.2 ± 0.6	12.9 - 13.4
Stature (m)	1.59 ± 0.09	1.54 - 1.62
Body Mass (kg)	48.0 ± 10.2	43.9 - 52.4
Maturity Offset (y)	-0.8 ± 0.9	-1.2 to – 0.4
Tanner Stage	3 ± 1	2 - 3
Predicted Age at PHV	14.0 ± 0.7	13.7 – 14.3
Σ 4 Skinfolds (mm)	30.2 ± 5.4	28.0 – 32.4
Body Fat (%)	17.6 ± 2.2	16.7 – 18.6
Fat Free Mass (kg)	39.6 ± 8.7	35.8 – 43.3
Training Years (y)	4.8 ± 2.2	3.9 - 5.7
Training Hours (hrs.p.week)	12.4 ± 2.7	11.4 - 13.7

644 Note: PHV = Peak Height Velocity; Skinfold sites used for the Σ4 skinfolds were the biceps, triceps, subscapular
645 and superiliac.
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Table 2: Differences in anthropometric and descriptive characteristics between those identified as pre- and mid-PHV, in highly trained youth soccer players.

	Mid-PHV (<i>n</i> = 11)		Pre-PHV (<i>n</i> = 10)		Difference		
	Mean ± SD	90% CL	Mean ± SD	90% CL	Sig.		Cohen's <i>d</i>
Age (yr)	13.4 ± 0.4	13.1 - 13.6	13.0 ± 0.7	12.5 - 13.4	<i>P</i> = 0.12	0.52	Moderate
Stature (m)	1.65 ± 0.08	1.60 - 1.69	1.52 ± 0.06	1.48 - 1.56	<i>P</i> < 0.05	1.83	Large
Body Mass (kg)	53.9 ± 10.7	48.2 - 60.5	41.6 ± 3.9	39.3 - 43.8	<i>P</i> < 0.05	1.53	Large
Tanner	3.2 ± 1.0	2.6 - 3.8	2.6 ± 0.5	2.3 - 2.9	<i>P</i> = 0.11	0.77	Moderate
Maturity Offset	-0.2 ± 0.7	-0.6 to 0.2	-1.5 ± 0.4	-1.7 to -1.3	<i>P</i> < 0.05	2.28	Large
Predicted Age at PHV	13.6 ± 0.6	13.2 - 13.9	14.4 ± 0.5	14.1 - 14.7	<i>P</i> < 0.05	1.45	Large
Σ Skinfolds (mm)	28.9 ± 4.2	26.6 - 31.4	31.6 ± 6.5	27.6 - 35.4	<i>P</i> = 0.26	0.49	Small
Body Fat (%)	17.2 ± 1.7	16.2 - 18.2	18.1 ± 2.6	16.5 - 19.8	<i>P</i> = 0.32	0.47	Small
Fat Free Mass (kg)	44.7 ± 9.3	39.2 - 50.2	34.0 ± 2.7	32.3 - 35.6	<i>P</i> < 0.05	1.62	Large
Training Years	3.7 ± 2.0	2.5 - 4.9	5.9 ± 1.8	4.8 - 6.9	<i>P</i> < 0.05	1.16	Large
Training Hours (per week)	12.3 ± 1.4	11.5 - 13.1	12.5 ± 3.7	10.3 - 15.1	<i>P</i> = 0.85	0.07	Trivial

Note: PHV = Peak Height Velocity; Skinfold sites used for the Σ4 skinfolds were the biceps, triceps, subscapular and superiliac.

Table 3: Differences between those identified as pre-PHV and mid-PHV, in measures of cardio-respiratory fitness during the maximal graded exercise and during both absolute (8km·h⁻¹) and relative (80% and 95% GET) submaximal running speeds, using different methods for normalizing VO₂.

	Mid-PHV (<i>n</i> = 11)		Pre-PHV (<i>n</i> = 10)		90% CI of Differences			Between Group Differences		
	Mean ± SD	90% CL	Mean ± SD	90% CL	Lower	Upper	Sig.	Cohen's <i>d</i>		Qualitative Descriptor
VO _{2peak} (L·min ⁻¹)	3.09 ± 0.53	2.80 - 3.42	2.50 ± 0.29	2.31 - 2.67	-0.96	-0.25	<i>P</i> = 0.005	1.38	Large	Very Likely
VO _{2max} (mL·kg BM ⁻¹ ·min ⁻¹)	58.6 ± 5.7	55.3 - 61.6	60.2 ± 4.9	57.3 - 63.2	-2.4	6.1	<i>P</i> = 0.49	0.30	Small	Unclear
VO _{2peak} (mL·kg FFM ⁻¹ ·min ⁻¹)	70.1 ± 7.3	65.8 - 74.3	73.5 ± 5.4	70.1 - 76.9	-1.4	8.3	<i>P</i> = 0.24	0.56	Moderate	Unclear
VO _{2peak} (mL·kg ^{-0.67} ·min ⁻¹)	214.9 ± 21.0	202.5 - 227.3	205.6 ± 17.3	194.9 - 216.3	-23.9	5.3	<i>P</i> = 0.29	0.50	Moderate	Unclear
VO _{2peak} (mL·kg ^{-0.75} ·min ⁻¹)	156.4 ± 15.1	147.5 - 165.4	152.6 ± 12.5	144.9 - 160.4	-14.3	6.7	<i>P</i> = 0.54	0.28	Small	Unclear
Max V _E VO ₂	32.0 ± 4.1	29.8 - 34.7	30.7 ± 1.7	29.7 - 31.8	-4.2	1.1	<i>P</i> = 0.36	0.41	Small	Unclear
Velocity at GET (km·h ⁻¹)	11.4 ± 1.0	10.8 - 11.9	10.7 ± 0.8	10.2 - 11.2	-1.5	0.1	<i>P</i> = 0.10	0.77	Moderate	Likely
VO ₂ at GET (% VO _{2peak})	81.9 ± 4.9	79.2 - 84.6	82.3 ± 4.2	79.8 - 85.1	-3.4	4.2	<i>P</i> = 0.83	0.08	Trivial	Unclear
Running Economy										
VO ₂ @ 8km/h (mL·kg BM ⁻¹ ·min ⁻¹)	39.6 ± 4.2	37.3 - 41.9	39.4 ± 4.6	36.7 - 42.4	-3.9	3.4	<i>P</i> = 0.94	0.05	Trivial	Unclear
VO ₂ @ 8km/h (mL·kg FFM ⁻¹ ·min ⁻¹)	47.8 ± 4.6	45.0 - 50.5	48.1 ± 5.4	44.8 - 51.5	-3.4	4.2	<i>P</i> = 0.87	0.08	Trivial	Unclear
VO ₂ @ 8km/h (mL·kg ^{-0.67} ·min ⁻¹)	146.9 ± 16.3	137.3 - 156.5	134.7 ± 16.5	124.5 - 144.9	-24.5	0.2	<i>P</i> = 0.11	0.78	Moderate	Likely
VO ₂ @ 8km/h (mL·kg ^{-0.75} ·min ⁻¹)	106.8 ± 11.3	100.1 - 113.6	100.0 ± 12.0	92.5 - 107.4	-15.7	1.9	<i>P</i> = 0.20	0.62	Moderate	Likely
V _E VO ₂ @ 8km·h ⁻¹	26.2 ± 2.4	24.8 - 27.6	27.6 ± 1.6	26.7 - 28.8	-0.4	3.2	<i>P</i> = 0.14	0.69	Moderate	Likely
RER @ 8km·h ⁻¹	0.89 ± 0.04	0.86 - 0.91	0.90 ± 0.03	0.88 - 0.92	-0.02	0.04	<i>P</i> = 0.52	0.28	Small	Unclear
%HRmax @ 8km·h ⁻¹	77.1 ± 4.9	74.4 - 79.7	77.9 ± 5.4	74.7 - 81.1	-3.3	5.3	<i>P</i> = 0.70	0.15	Trivial	Unclear
% VO _{2peak} @ 8km·h ⁻¹	67.7 ± 4.5	65.0 - 70.3	65.6 ± 7.7	61.2 - 70.4	-7.4	3.3	<i>P</i> = 0.46	0.33	Small	Unclear
VO ₂ @ 80%GET (mL·kg BM ⁻¹ ·min ⁻¹)	43.7 ± 5.1	41.0 - 47.0	42.2 ± 3.8	39.7 - 44.3	-5.4	1.9	<i>P</i> = 0.43	0.33	Small	Unclear
VO ₂ @ 80%GET (mL·kg FFM ⁻¹ ·min ⁻¹)	52.8 ± 5.9	49.3 - 56.3	51.7 ± 4.6	48.8 - 54.6	-5.2	2.9	<i>P</i> = 0.64	0.22	Small	Unclear
VO ₂ @ 80%GET (mL·kg ^{-0.67} ·min ⁻¹)	162.2 ± 19.2	150.9 - 173.6	144.5 ± 13.4	136.2 - 152.8	-30.3	-5.1	<i>P</i> = 0.025	1.12	Large	Very Likely
VO ₂ @ 80%GET (mL·kg ^{-0.75} ·min ⁻¹)	118.0 ± 13.5	110.0 - 126.0	107.3 ± 9.8	101.2 - 113.4	-19.7	-1.7	<i>P</i> = 0.053	0.95	Large	Likely
V _E VO ₂ @ 80%GET	26.9 ± 2.1	25.6 - 28.1	27.9 ± 1.5	27.0 - 28.8	-0.5	2.5	<i>P</i> = 0.28	0.55	Moderate	Unclear
RER @ 80%GET	0.90 ± 0.03	0.88 - 0.92	0.91 ± 0.03	0.89 - 0.92	-0.01	0.03	<i>P</i> = 0.45	0.33	Small	Unclear
%HRmax @ 80%GET	82.4 ± 4.9	79.1 - 84.8	82.4 ± 4.1	79.8 - 84.7	-3.8	4.1	<i>P</i> = 0.99	0.00	no effect	Unclear
% VO _{2peak} @ 80%GET	74.8 ± 5.6	71.4 - 78.0	70.2 ± 4.5	67.4 - 72.8	-8.7	-0.2	<i>P</i> = 0.049	0.67	Moderate	Likely
VO ₂ @ 95%GET (mL·kg BM ⁻¹ ·min ⁻¹)	50.6 ± 5.0	47.9 - 53.4	48.6 ± 4.2	46.0 - 51.0	-5.8	1.7	<i>P</i> = 0.33	0.43	Small	Unclear
VO ₂ @ 95%GET (mL·kg FFM ⁻¹ ·min ⁻¹)	61.3 ± 5.6	58.0 - 64.6	59.6 ± 5.3	56.3 - 62.9	-5.8	2.4	<i>P</i> = 0.49	0.32	Small	Unclear
VO ₂ @ 95%GET (mL·kg ^{-0.67} ·min ⁻¹)	188.3 ± 19.7	176.7 - 199.9	166.6 ± 15.2	157.2 - 176.0	-35.1	-8.3	<i>P</i> = 0.011	1.29	Large	Very Likely
VO ₂ @ 95%GET (mL·kg ^{-0.75} ·min ⁻¹)	137.0 ± 13.5	129.0 - 144.9	123.7 ± 11.1	116.8 - 130.6	-22.7	-3.9	<i>P</i> = 0.024	1.13	Large	Very Likely
V _E VO ₂ @ 95%GET	27.5 ± 2.6	25.9 - 28.9	27.8 ± 2.3	26.5 - 29.1	-1.6	2.3	<i>P</i> = 0.77	0.12	Trivial	Unclear
RER @ 95%GET	0.93 ± 0.04	0.91 - 0.95	0.93 ± 0.04	0.90 - 0.96	-0.03	0.03	<i>P</i> = 0.99	0.00	no effect	Unclear
%HRmax @ 95%GET	88.7 ± 4.3	86.0 - 91.0	89.2 ± 3.9	86.5 - 91.4	-2.9	4.0	<i>P</i> = 0.77	0.12	Trivial	Unclear
% VO _{2peak} @ 95%GET	86.6 ± 5.3	82.9 - 89.6	81.1 ± 7.4	75.9 - 85.0	-11.4	-0.1	<i>P</i> = 0.06	0.85	Large	Likely
VO₂ Kinetics										
low - mod <i>tau</i> (s)	22.0 ± 8.1	18.2 - 27.4	16.4 ± 5.6	13.6 - 20.0	-11.7	-0.06	<i>P</i> = 0.08	0.80	Large	Likely
low - mod amplitude (L·min ⁻¹)	1.91 ± 0.43	1.68 - 2.15	1.52 ± 0.19	1.41 - 1.63	-0.66	-0.14	<i>P</i> = 0.016	1.17	Large	Very Likely
mod - sev <i>tau</i> (s)	78.8 ± 61.0	47.1 - 119.6	47.6 ± 23.7	34.6 - 64.7	-73.4	2.9	<i>P</i> = 0.15	0.67	Moderate	Likely
mod - sev amplitude (L·min ⁻¹)	0.56 ± 0.21	0.44 - 0.69	0.44 ± 0.24	0.31 - 0.59	-0.32	0.07	<i>P</i> = 0.22	0.53	Moderate	Likely
Yo-Yo IRI (m)	1682 ± 304	1512 - 1855	1572 ± 360	1364 - 1798	-402	188	<i>P</i> = 0.49	0.33	Small	Unclear

666 Note: $\text{VO}_{2\text{peak}}$ = peak oxygen consumption; GET= gaseous exchange threshold; $V_E\text{VO}_2$ = ventilatory equivalent; RER= respiratory exchange ratio; HR_{max} = maximal heart rate;
667 τ = time constant; Yo-Yo IR1= Yo-Yo intermittent recovery test level 1.